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DAILY EGG PRODUCTION METHOD SURVEY

Prepared for

WORKSHOP ON ENHANCING STOCK ASSESSMENTS OF PACIFIC SARDINE IN

THE CALIFORNIA CURRENT THROUGH COOPERATIVE SURVEYS

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I. Introduction and Background of Daily egg production method (DEPM) survey

1.1 Theoretical basis and assumptions (concept, absolute biomass, abundance index; conditions under which method works)

The daily egg production method (DEPM) was developed by the staff of the Coastal Fisheries Resources Division of the Southwest Fisheries Center in the early 1980s for Northern anchovy off California to estimate the absolute spawning biomass off central and southern California (Lasker 1985).

The basic concept of the DEPM is that the spawning biomass can be computed as the ratio of the total number of eggs produced per day and the number of eggs to be released by the adult fish per unit fish weight per day. In order to apply the DEPM, the following criteria must be met: fish has to be a multiple spawner and we can measure when females spawn (the day of trawling, one, two or three days prior to trawling, noted as day-0, day-1,day-2 and day-3 females), and the fish has easily identifiable pelagic eggs distributed in the upper layers of the ocean, e.g. 70 m depth, that can be captured by a plankton net, like CalVET net tows. Spawning and nonspawning adults must be equally available to a trawl or similar sampler. The entire spawning area should be encompassed by the cruise. This method has been applied to anchovy, sardine, sprat, mackerel, and horse mackerel in 16 locations around the world since 1980 (Stratoudakis et al. 2006). The application was extended to demersal species, like snapper in New Zealand (Zeldis and Francis, 1998) and hake off Spain (Murua et al. 2009).

1.2 Objectives of application (what stock assessment input does method produce)

Since the recovery of Pacific sardine in the mid-1980s, the initial objective of the DEPM was to estimate the spawning biomass starting in 1986. The long time series of each biological variable of fish stocks provide us with insights into the biology of the species through time to best understand the population and possibly prevent its collapse in the

future. Starting in 1996, the DEPM spawning biomass has been used as one of the fishery-independent time series for stock assessment (Hill et al. 2009).

1.3 Pros and Cons of method (advantages and disadvantages)

The chief advantages of this egg production method are: 1) Eggs are non-evasive, thus the catchability is not a problem. 2) It produces estimates of egg production, egg mortality, reproductive parameters, and spawning biomass with the measurements of precision, e.g. the coefficient of variation (CV). 3) It requires only a single cruise with one or two ships for around a month (Alheit 1993) and 4) Both ichyoplankton and trawl samples include data of other species which can be used to understand the interaction among species together with oceanographic variables and thus the ecosystem in the California Current.

The major disadvantages of the method are: 1) eggs are patchy with short duration (\sim 3 days). This requires a well designed survey or a large number of samples to achieve estimates of high precision. Eggs need to be classified into different developmental stages. A temperature-dependent developmental rate model was necessary to convert stage to age in order to estimate egg production at age $zero(P_0)$. 2) Two adult parameters: the fecundity and the daily spawning fraction, the proportion of mature females spawning per day, require intensive laboratory work and large number of trawls to achieve reliable estimates(Lasker 1985) and 3) Application of the DEPM to migratory species may be more complicated than non-migratory species, like sardine verses anchovy.

II. Design for DEPM Survey in the California Current (current design and potential design enhancements)

2.1 Spatial and temporal coverage

The spatial coverage varies with the population size. In the 1980s during the resurgence of Pacific sardine population, the survey areas were much inshore between San Pedro and Point Conception, i.e. the August survey in 1986 and July survey in 1987. In the May 1988 survey, the survey area extended north to San Francisco. As the population expanded and more sardine eggs were shown in spring than summer, the surveys, starting in 1994, were conducted in late-March to early May and occupied the area from San Diego to San Francisco for most years except the April 2006 and 2008 California Current Ecosystem (CCE) surveys which covered the area from San Diego to the US-Canada border with the farthest stations 360 nm from the shore off southern California (Figure 1).

2.2 Statistical sampling protocols

The ichthyoplankton-trawl DEPM surveys were conducted in 1986-1988 by the California Department of Fish and Game (CDFG), in 1994 by Mexico (INP), CDFG and Southwest Fisheries Science Center (SWFSC), and in 2004-present by SWFSC. For the ichthyoplankton surveys, a fixed-station survey design was used prior to 1997. The CV of P₀ ranged from 0.22 (684 tows in 1994) to 0.83 (330 tows in 1988). Beginning in 1997,

an adaptive allocation survey design was implemented using the underway egg sampler (CUFES) (Checkley et al. 1997) to locate the additional CalCOFI vertical egg tows (CalVET). The CV of P_0 was improved, ranging from 0.17 to 0.42 with fewer than 200 CalVET tows. Thus, the adaptive allocation surveys are efficient for the CalVET tows. However, the number of total CUFES collections ranged from 600 to near 1000 during each survey.

For each DEPM-CalCOFI survey, CalVET and Bongo tows were taken at pre-determined stations. Only during the DEPM sardine surveys started in 1997, is an adaptive allocation survey procedure (Lo et al. 2001) used, i.e. additional CalVET tows were taken at 4 nm intervals on each line after the egg density from each of two consecutive CUFES samples exceeded 1 egg/min, and CalVET tows were stopped after the egg density from each of two consecutive CUFES samples was less than 1 egg/min. One egg/min is equivalent to two to seven eggs/CalVET tow, depending on the degree of water mixing. The threshold value was reduced to 1 egg/min from 2eggs/min used prior to 2002 to increase the size of the high density area and, subsequently, to increase the number of CalVET samples. The threshold value may vary for surveys in different areas due to the difference of egg densities. For example, during July 2003 and July 2004 Pacific NW surveys the threshold value was set at 0.5 eggs/min (Lo et al. 2010). In the future, the allocation of transect lines and stations can be improved by incorporating both the sea surface temperature and Chlorophyll-a concentration habitat model (Zwolinski et. al. in prep) in addition to the CUFES on-site catches. The habitat model can also be used to define the location of the northern boundary of the survey area.

Although the ichthyoplankton survey was conducted yearly, adult fish samples were collected only in 1986-1988, 1994, and 2004-present. A few opportunistic trawl samples were taken in 1997, 2001, and 2002. During 1986-1988 and 1994 fish were caught by several types of gear (purse seine, mid-water trawl, and hook and line) and since 1994 by mid-water or surface trawls. All sampling was conducted in the night when the weather allowed. In recent years the number of trawl samples that contained mature females has fluctuated around 14 with a range of 7 in 2006 to 29 in 2009. Recently, 3-5 trawls are conducted each night, to potentially increase the number of positives, and in areas of no/low sardine egg presence as well as areas of high sardine egg concentrations, to ensure capturing a broad range of the mature sardine population.

2.3 Data collection and treatment

Egg and larval specimens were brought to the Fisheries Resources Division (FRD) larval laboratory for sorting, assignment of development stages for eggs and length measurement for larvae. The final data were entered into the data base together with station information. The age of eggs was assigned based on a temperature-dependent-developmental-rate model.

Up to 50 sardines were randomly sampled from each trawl with more than 75 fish, or all otherwise. If necessary, additional mature females were collected to obtain 25 mature females per trawl to estimate reproductive parameters. Each fish was sexed, standard

length (mm) and weight (g) were measured, otoliths were removed for aging, tissue was preserved in 95% ethanol for genetics, and ovaries were removed and preserved in 10% neutral buffered formalin. Each preserved ovary was blotted and weighed to the nearest milligram in the laboratory. A piece of each ovary was removed and prepared as hematoxylin and eosin (H&E) histological slides. All slides were analyzed for oocyte development, atresia, and postovulatory follicle age to assign female maturity and reproductive state and to identify ovaries for fecundity estimations (Macewicz et al. 1996).

2.4 Analytical procedure (including variance estimations)

The model for the DEPM is to estimate two parameters: the daily rate of egg production per unit area at age 0 (P_0) from the ichthyoplankton samples and the daily specific fecundity (number of oocytes/gram biomass/day) from adult sardine from the trawl samples. The ratio of these two quantities is the estimate of the spawning biomass.

The spawning biomass was computed as

$$B_s = \frac{P_0 A C}{RSF/W_f}$$
 [1]

where A is the survey area in unit of 0.05 m², S is the fraction of mature females spawning per day, F is the batch fecundity (number of eggs per spawn per mature female), R is the fraction of mature female fish by weight (sex ratio), W_f is the average weight of mature females (g), and C is the conversion factor from grams (g) to metric tons (mt). P_0A is the total daily egg production in the survey area, and the denominator (RSF/W_f) is the daily specific fecundity (number of eggs/population weight (g)/day).

The variance of the spawning biomass estimate (\hat{B}_s) was computed from the Taylor expansion and in terms of the coefficient of variation (CV) for each parameter estimate and covariance for adult parameter estimates (Parker 1985):

$$VAR\left(\hat{P}_{s}\right) = \hat{B}_{s}^{2} \left[CV\left(\hat{P}_{0}\right) + CV\left(\hat{V}_{f}\right) + CV\left(\hat{S}\right) + CV\left(\hat{R}\right) + CV\left(\hat{F}\right) + 2COVS \right] [2]$$

Where COVS is a term including covariance between adult parameter estimates, and P_0 is the intercept of the egg mortality curve: $P_{age} = P_0 \exp(z^*age)$. Each adult parameter was estimated by a ratio estimator, e. g. the sex ratio is equal to the total female weight divided by the total fish weight based on data from all positive trawls.

During 1995-2001, when trawls samples were few or lacking, an overall average of the spawning fraction during 1986–94 and estimates of other adult parameters in 1994 were used to estimate daily specific fecundity (number of eggs/gram weight). In 2003, the estimates of adult reproductive parameters from 2002 were used. Since 2004, a full-scale survey has been conducted each year to estimate the spawning

biomass of Pacific sardine.

The survey area was post-stratified into two regions based on the presence of sardine eggs: region 1 (high density area) and region 2 (low density area), Therefore equation (1) can be applied to the whole survey area and/or to each of the two regions depending on the availability of data. For the female spawning biomass (fs. biomass), one of the inputs to the stock assessment, the sex ratio (R), was excluded from equations (1) and (2).

III. Lessons Learned from Application

3.1 Method conditions met? (biological, ecological, oceanographic)

Most conditions required to use the DEPM are met: Pacific sardine is a multiple spawner. Eggs are easy to identify, are distributed predominately in upper 50 m depth (Ahlstrom 1959) and almost all are in the upper 70 m depth. Trawl samples caught spawning and nonspawning adults. Because the females that spawn during the trawling (day-0 females) were more aggregated, they were replaced in the spawning biomass computation by the average of day-1 and day-2 females to lessen the bias. The only condition that may not have been met in some recent years is the requirement for the survey area to encompass the total spawning area. However, the standard DEPM survey area encompasses most of the spawning area in April. A small portion of adult sardine may reside or migrate from south into the area between 38-43 N in April, and thus the spawning biomass may be slightly underestimated in some years. For example, in 2006 around 10% of the spawning biomass was outside the standard DEPM survey area. Estimates of migration rates are useful if the survey area is restricted to the standard DEPM survey area.

The evidence from most April surveys during 2003-2008 outside the standard DEPM survey area indicated that most adult sardines were observed south of 38N in the standard DEPM survey area. During a set of four surveys covering the area of 42-48N in July 2003, March, and July 2004 and March 2005 (Lo et al. 2010), most fish caught in March, 2004 and 2005 were young and recruit sardines (<200mm), that resulted from the strong 2003 year class (Lo et al. 2010). In two coast-wide surveys, in April 2006 (30-51N) and 2008 (30-48N) (Lo at al. 2007, McClatchie 2009), adult sardine and sardine eggs were observed between 38-41 N in April 2006 but only one sardine of 240 mm and few eggs were caught close to 37 N in April 2008, partially due to no strong incoming year classes (Lo et. al. 2009). Therefore, in April, we only expect very low abundance of adult sardine north of 43 N. Some adult sardines may reside north of the routine DEPM survey area up to 43 N, as observed in April 2006 survey. However, during the current 2010 coast- wide survey, sardines were caught only south of 38 N, the standard DEPM survey area. If adult fish in the area of 38-43 N are only migratory fish, the survey strategy should start from the northern line of the standard DEPM survey area heading south to have a better chance of catching adult sardine migrating to the north after their spawning off southern or central California. (McClatchie 2009). Nevertheless, the possible underestimation of the spawning biomass is not due to the DEPM methodology itself, rather it is due to the survey area not always encompassing the entire spawning area.

3.2 Operation conditions (Staffing and funding)

For the DEPM survey, we used the 2010 survey as an example to compute the cost of ship time, data processing and data analyses. In 2010, we conducted a coast-wide survey, including two vessels: R/V Miller Freeman (M.F.) to cover the standard DEPM survey area from San Diego to San Francisco on April 2-20 and a charted commercial vessel F/V Frosti to cover the area from San Francisco to the US-Canadian border on March 26-April 28th. In addition, the CalCOFI survey was conducted after the coast-wide survey to cover the regular CalCOFI area from San Diego to Point Conception from April 26-May 17th. For the DEPM work, a total of five DEPM scientists joined the survey in the northern area, six DEPM scientists joined the standard DEPM area and two joined the CalCOFI survey. Our estimated total cost for the north survey, standard DEPM and CalCOFI was \$420,000, \$560,000 and \$470,000 respectively. The total costs included the ship time, regular salary for the preparation prior to, during, and after the survey, laboratory work, data analyses after the surveys and overtime during the surveys (Table 1). Thus the cost of DEPM survey depends on the areal coverage and whether the CalCOFI survey is conducted during the peak spawning time of Pacific sardine and included in the DEPM spawning biomass computation. The cost ranges from half million to 1.4 million. All the three surveys collect data of all species available and thus do benefit not only the understanding of the Pacific sardine but the whole ecosystem of the survey area.

Table 1. The estimated costs for 2010 coast-wide survey and April-May CalCOFI survey: the regular and over time salary, ship time (\$17k/day and \$8k/day for M. Freeman and Frosti respectively) and sea going staff, some of whom participated in one or two legs of the cruises.

2010 Survey DEPM	regular + over time	Ship time	equipment shipping	Total	person- day at sea	persons	Ship days
North (Frosti)	\$127,126	\$280,000	\$8,000	\$415,126	123	5	35
Standard DEPM (M.F.)	\$177,072	\$374,000	\$8,000	\$559,072	99	6	22
CalCOFI	\$88,970	\$374,000	\$8,000	\$470,970	44	2	22
Total	\$393,167	\$1,028,000	\$24,000	\$1,445,167			

3.3 Past peer-review advice for improvement (e.g. STAR)

The 2009 May STAR panel on surveys of Pacific sardine recommended the following for the longer-term research priorities:

- Efforts should be made to increase the number of samples of adults;
- A hierarchical modeling approach (a.k.a. random effects modeling) should be adopted for estimation of spawning rate parameters;

• The use of day-2 and day-3 follicles should be explored as a way to estimate the spawning fraction;

- The relative abundance of fish deeper than can be sampled by the trawls should be determined (perhaps by means of acoustics) and an evaluation conducted whether these fish represent a significant source of uncertainty;
- Additional sources of adult fish samples should be explored.

A hierarchical modeling (HM) approach was used to estimate the spawning fraction based on the average of day-1 and day-2 females for years when trawl samples were available. The point estimates of the HM and those of the original methods are similar while the most CVs of the HM estimates were lower than those of the original methods. For years, 997, 2001 and 2002 with few trawls, CVs were improved for 2001 and 2002. To increase the number of trawls, the STAR panel suggested that commercial fishing vessels are a potential source of adult samples, and cooperative research with the fishing industry would be worth pursuing. The latter may require an EFP from the Council, and the project would be a good candidate for NMFS cooperative research funding.

IV. Workshop Recommendations for Surveys to Enhance Stock Assessments

4.1 Oportunities for collaboration

A possible tagging experiment of sardine using electronic acoustic receivers used for salmon inshore off NW may be conducted by the Northwest Fisheries Science Center. If successful, the tagging operation will be expanded offshore to estimate the migration rates of Pacific sardine.

The coast-wide July survey can be expanded to the British Column area. Thus we need the participation of Canadian vessels and sea-going personnel. Both ichthyoplankton tow nets and CUFES need to be loaned to Canadian vessels.

Industry could provide in-kind vessels like F/V Frosti to do a trawl-ichthyoplankton-acoustic survey during the coast-wide survey in April and July, in particular to obtain adult samples for reproductive output as recommended by the 2009 May STAR panel (see section 3.3).

4.2 Linkage to other methods

The time series of the spawning biomass, and biomass from the acoustic surveys in April and/or July can be established for many years like was done for South Africa anchovy from 1984-1994 (Hampton, 1996; van der Ligen and Huggett, 2003) and the Altanto-Iberian sardine (Maria Manuel Angelico, pers. Commu. Egg production workshop in March 10-13, 2010, Athens, Greece). The spawning biomass of South Africa anchovy was used to calibrate relative estimates of anchovy biomass made using hydroacoustic methods. While the time series of DEPM spawning biomass and acoustic biomass matched well for anchovy off South Africa, this was not the case for the Altanto-Iberian

sardine. Namely, the increasing trend of DEPM spawning biomass in the past decade was not depicted in the results obtained by the acoustic estimates of biomass.

The time series of the spawning biomass, and biomass from the acoustic and aerial surveys, can be established for many years for comparison purposes. To be in line with the ecosystem management, the current aerial survey would best be broadened to collect data of other species as done by the spotter pilot logbook survey off California many years ago.

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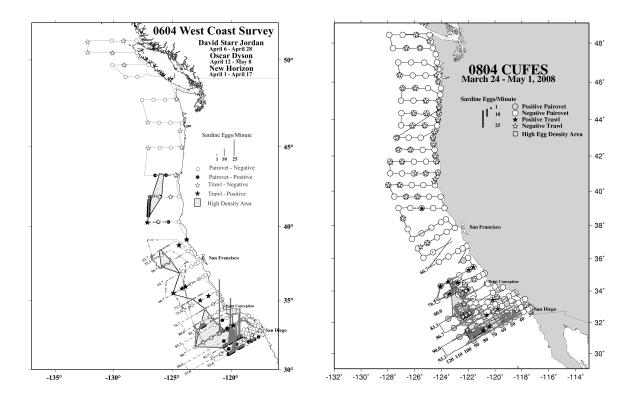


Figure 1. Location of sardine eggs collected from CalVET (circle) and from CUFES (sticks) and trawl (star). Solid symbols are positive catch. High density area is shaded area for April cruise in 2006 and 2008.